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Further Evidence of Male Antennal Glands in Aphelinidae: The Case of *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae)

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Abstract.—Ultrastructural investigations of the elongated male club, antennomere 6, in *Aphytis melinus* DeBach have shown that a small oval ventral area, bearing numerous minute setae, is not a sensory complex but rather the release site of a glandular complex with unicellular unit type 1. This finding, combined with behavioral observations reported in the literature, strongly indicates that the secretion induces sex recognition during pre-coital phase. This morpho-functional interpretation is discussed in other aphelinids exhibiting similar structures on different antennomeres.

In the last few years, some peculiar antennal structures of parasitoid hymenopterans have received increasing interest, and have revealed new aspects for functional morphology, biosystematics, and phylogeny (Isidoro *et al.* 1996). In fact, the male antennae, which were previously believed to be only sensory appendages, have instead been demonstrated as also having a secretory function through epidermal glands involved in courtship behavior (Bin *et al.* 1997). The secretory function has been reported in families Eulophidae (Dahms 1984), Scelionidae (Bin and Vinson 1986), Platygastridae (Isidoro and Bin 1995), Ichneumonidae (Isidoro *et al.* 1997), Diapriidae (Romani *et al.* 1997; Sacchetti *et al.* 1997) and Eucoilidae (Isidoro *et al.* submitted).

Male antennal glands were reported for the first time in *Encarsia asterobemisiae* Viggiani et Mazzone (Pedata *et al.* 1995), a species supplied with two peculiar ventral features, respectively on antennomeres four and five, which likely appear to be used during pre- and post-coital phases (Viggiani and Laudonia 1989). In another aphelinid, *Aphytis melinus* DeBach, an important worldwide biocontrol agent of citrus scales, the courtship behavior was also described stressing the importance of antennation during the precoital phase (Gordh and DeBach 1978). The occurrence of this behavior, along with the presence of a "specialized sensory area" on the ventral side of the male elongated club, has prompted an ultrastructural study of this "small oval area or plate bearing numerous minute setae" (Rosen and DeBach 1979).

This study proves that in *A. melinus* such an area, present on A6 in the form of an elongated club, is the release site of adjacent integumentary glands. In addition, for the first time in hymenopterans, glands are reported on the apical antennomere.

MATERIALS AND METHODS

A colony of *A. melinus*, laboratory reared on *Aonidiella aurantii* (Maskell), was provided by the Entomology Department of the University of California (Riverside).

For scanning electron microscopy (SEM) observations, 10 males, newly emerged and anaesthetized in CO_2, were beheaded and immediately immersed in 50% ethanol water solution and kept overnight at 4°C. After dehydration in a graded ethanol series, the heads with antennae were critical point dried in a Balzers Union CPD

020 unit, gold coated in a Balzers Union SCD 040 unit, and finally examined with a Philips XL 30.

For transmission electron microscopy (TEM) observations, 10 males were anaesthetized in CO2 and immediately immersed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer + 5% sucrose, pH 7.2–7.3. The apical antennomeres were detached to aid fixative penetration, and left at 4°C for 2h. After rinsing overnight in a cacodylate buffer, the specimens were postfixed in 1% osmium tetroxide at 4°C for 1h and rinsed in the same buffer. Dehydration in a graded ethanol series was followed by embedding in Epon-Araldite with propylene oxide as a bridging solvent. Thin sections were taken with a diamond knife on a L.K.B. "Nova" ultramicrotome, and mounted on collodium-coated 50 mesh grids. Finally, the sections were investigated with a Philips EM 400T, after staining with uranyl acetate (20 min, room temperature) and lead citrate (5 min, room temperature).

RESULTS

The geniculate antennae of male *A. melinus* consist of six antennomeres. The apical antennomere, A6 or club, is elongated and bears an oval area on the proximal ventral side which is the release site structure (RSS) of the integumentary glands (Fig 1a). This area is covered by minute, non-socketed microtrichia and bordered by one row of socketed trichoid sensilla (Fig 1b); while the former are not innervated the latter are provided of one mechanosensory neuron. SEM observations of the ventral side of A6 show the oval area slightly depressed and partially cut off from the surrounding club portion by indistinct grooves.

Serial longitudinal and cross sections of the apical antennomere reveal a well developed glandular epithelium adhering to the internal wall of the oval area (Fig 2a). This glandular complex consists of numerous, unicellular secretory units varying in size and shape. Each glandular cell has a large, round and regularly shaped nucleus which is often located in the basal part of the cell (Fig 2b). Chromatin is not abundant and most of it is apposed to the nuclear membrane. The perinuclear region of the cytoplasm contains mitochondria with conventional cristae and abundant free ribosomes while few signs of granular or smooth endoplasmic reticulum were observed. The basal plasma membrane of the cell has deep, irregular invaginations forming a lacunar system. The apical cell membrane is surrounded by densely packed microvilli, delimiting a narrow extracellular space. The cuticle associated with the glandular epithelium is pierced by numerous tiny pores randomly distributed. The secretory apparatus underneath each pore is formed by a spherical chamber, from which numerous cuticular filaments radiate (Fig 2c). These filaments, apparently a specialization of the external epicuticle, have a tubular structure and extend deeply in the extracellular space between the microvilli of the apical cell membrane.

DISCUSSION

The "small oval area or plate bearing numerous minute setae" on the ventral side of male club in *A. melinus* (Rosen and DeBach 1979) is not a "specialized sensory area" but the release site of epidermal glands. These glands are unicellular secretory units belonging to the type 1 gland cell (Noirot and Quennedey 1974, 1991; Quennedey 1998). The cytological features of the secretory cells do not allow us to attempt an interpretation on the nature of the secretion which in other hymenopterans acts on contact (Isidoro *et al.* 1996) or is volatile (Felicioli *et al.* 1998). The peculiar releasing apparatus consists of numerous pores so tiny that neither the external openings nor the material secreted can be seen with SEM, contrary to what has been reported for other parasitoids (Bin and Vinson 1986; Isidoro and Bin 1995). The

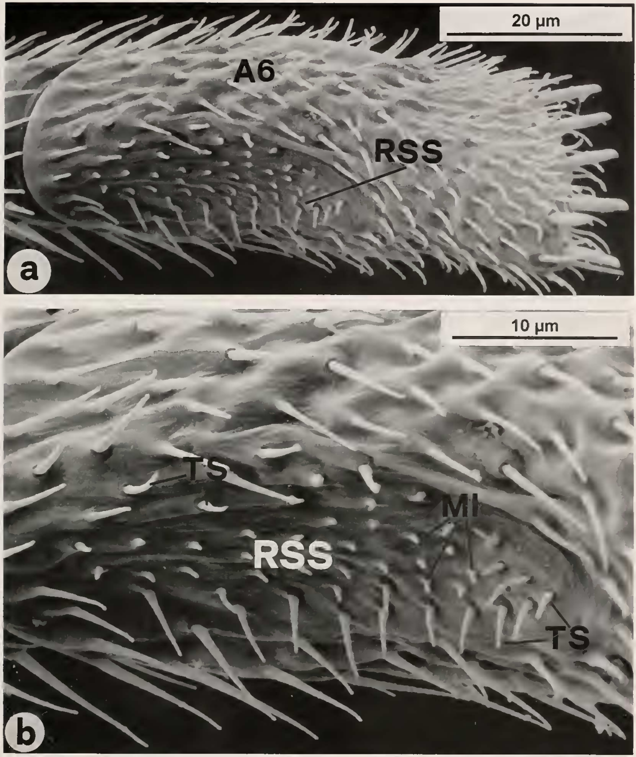

Fig. 1. *Aphytis melinus* male: a) ventro-lateral view of antennomere 6 (A6) showing the release site structure (RSS); b) detail of the RSS covered by numerous microtrichia (MI) and bordered by a single row of tactile setae (TS).

conceivable function of no-socketed microtrichia is that of increasing the release surface while that of socketed trichoid sensilla is that of perceiving tactile stimuli.

Encarsia asterobemisiae has two glandular complexes, respectively on A3 and A4, belonging to the same type 1 but different in cytological characteristics and release site

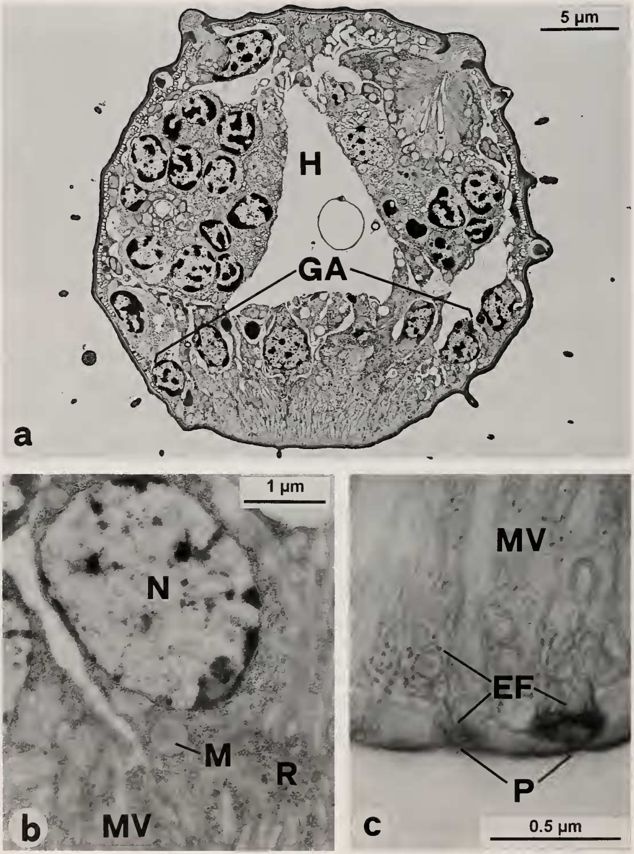

Fig. 2. *Aphytis melinus* male: a) cross section of antennomere 6 at about half level showing the extension of the glandular area (GA) on the ventral side; b) perinuclear detail of a secretory cell; c) apical detail of a secretory cell showing the tiny cuticular pores (P). N, nucleus; EF, epicuticular filaments; H, haemocele; M, mitochondrion; MV, microvilli; R, ribosomes.

features. This could indicate a difference in composition and role of the relative secretions (Pedata *et al.* 1995). From these two ascertained cases it appears that the so called sensorial complexes described in other aphelinids need to be investigated to define their real nature. Morphological and behavioral observations strongly suggest in fact that glands are present on different antennomeres involving the scape (A1), from one to 3 intermediate antennomeres (A3 to A5) or the apical antennomere (A6). Some examples are as follows:

On A1 of *Physcus testaceus* Masi there are special structures which could be related to a gland (Viggiani *et al.* 1986) and something similar occurs in *Pteroptryx chinensis* (Howard) (Viggiani and Ren 1993). Numerous species of *Encarsia* Foerster have one up to three antennomeres, A3–A5, which may or may not be noticeably enlarged, and ventrally provided of one specialized structure or two different ones. When two or more antennomeres are glanded the release sites are of two types; furthermore, one enlarged antennomere does not necessarily bear a specialized structure, as A3 in the case of *E. asterobemisiae* (Pedata *et al.* 1995). Therefore, some of the following examples having two or three enlarged antennomeres may need to be confirmed: A3 in *Encarsia albiscutellum* (Girault) (Viggiani 1985), A4 in *Encarsia aleurotubae* Viggiani, *E. herndoni* (Girault) (Viggiani 1987), A3–A4 in *Encarsia olivina* (Masi) (Viggiani and Mazzone 1982), *E. gigas* (Tchumakova), *E. opulenta* Silvestri and *E. perniciosi* (Tower) (Viggiani and Laudonia 1989), A3–A5 in *Encarsia antiopa* (Girault) (Viggiani 1985).

As regards the apical antennomere the A6 of several species of *Aphytis* Howard, as illustrated by Rosen and DeBach (1979), have external features similar to those described for *melinus*, and therefore could have glands: *A. cochereaui* DeBach and Rosen, *A. fabresi* DeBach and Rosen, *A. chilensis* Howard, *A. columbi* (Girault), *A. paramaculicornis* DeBach and Rosen, *A. diaspidis* (Howard), *A. lingnanensis* Compere, *A. roseni* DeBach and Gordh, *A. pilosus* DeBach and Rosen.

Behavioral observations indicate that the antennation mechanism during mating is different in species having one or two glanded antennomeres. While the single release site seems to be logically related to the pre-coital phase, e.g. triggering sex-recognition, female stimulation or female sedation (Gordh and DeBach 1978; Viggiani *et al.* 1986), the presence of a second and different release site has been observed in species performing also a post-coital phase, e.g. *E. asterobemisiae* (Pedata *et al.* 1995).

These chemo-physical mechanisms can only partially explain the sexual isolation since there also are other chemicals involved in mating behavior. In some species of *Aphytis* a female sex pheromone (Rao and DeBach 1969) and a male aphrodisiac pheromone (Khasimuddin and DeBach 1975), whose sources are as yet unknown (Gordh and DeBach 1978), have in fact been reported. In another aphelinid, *Aphelinus asychis* Walker, the mate finding is mediated by a female trail sex pheromone deposited in a yet unknown way on the substrate while walking (Fauvergue *et al.* 1995).

As it is being shown in other parasitoid groups (Bin *et al.* 1997; Isidoro *et al.* submitted) the glandular nature of the antennal structures provides a new perspective to define the sex selection strategies of aphelinids as well as additional characters for taxonomy and phylogeny.

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